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CLASSIFICATION OF THE KLEBSIELLA-AEROBACTER GROUP WITH SPECIAL REFERENCE TO THE COLD-TOLERANT MESOPHILIC AEROBACTER TYPES

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SUMMARY: Aërobacter liquefaciens Grimes and Hennerty 1931 is a later illegitimate homonym of A. liquefaciens Beijerinck 1900. A. lipolyticus nom. nov. is proposed in its place. A detailed description is given. A. lipolyticus is widely distributed in nature and is important in dairy science. It usually fails to produce gas from lactose at 37°C, but produces it abundantly at 20-30°C. A. aërogenes with which it may be confused, produces gas from lactose at 37°C. A. lipolyticus differs from A. hibernicus by liquefaction of gelatin.

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Since Escherich (1885) published his paper on "Intestinal Bacteria" and Beijerinck (1900) described and named Aërobacter aërogenes, the classification of the taxa of the family Enterobacteriaceae has been a subject for investigation, discussion and review. The different viewpoints on the interrelationships of the genera Klebsiella and Aërobacter are discussed in the 7th edition (1957) of Bergey's Manual of Determinative Bacteriology, the International Bulletin of Bacteriological Nomenclature and Taxonomy founded in 1951 and in many papers published in the various journals dealing with bacteriology, microbiology and public health.

The Enterobacteriaceae Subcommittee (originally The Salmonella Subcommittee) and the Judicial Commission and their members collectively and individually have expressed their viewpoints in a series of papers in the International Bulletin.

Kauffmann and Edwards (1952) emended the description of Klebsiella to include nonmotile, nonliquefying, Gram-negative rods, fermenting lactose and mannitol, citrate positive and urea negative, or late and irregularly positive, and named two species, K. pneumoniae and K. rhinoscleromatis. They stated:

> "It is realized that no provision is made for the motile forms classified as Aërobacter. It is felt that present knowledge of these forms is insufficient to justify their placement in a definite group."

Edwards and Fife (1955) studied 856 cultures of Klebsiella and Aërobacter and stated that no single biochemical test or combination of tests sufficed clearly to distinguish the genus Klebsiella and A. aërogenes from cultures of A. cloacae, and that many aberrant and intermediate strains occurred. It was suggested that the nonmotile forms which do not liquefy gelatin be placed in the genus Klebsiella, and that Aërobacter be redefined as a motile liquefying group with A. cloacae as the type. With regard to motility, Brooke (1953) in his study of strains of A. cloacae and Klebsiella (all isolated from human sources, most of them from urine) noted that while all the Klebsiellae were nonmotile, a number of A. cloacae strains were also nonmotile.

Cowan (1954) reviewed the names of the coliform bacteria and asked the Judicial Commission to consider whether Aërobacter Beijerinck 1900 should be placed in the list of nomina generica rejicienda. The Commission should also be asked to determine whether a newly defined genus Aërobacter (not Aërobacter Beijerinck) should be conserved with the type A. cloacae Jordan, or with a redefined (motile) species A. aërogenes, or whether the genus Cloaca Castellani and Chalmers 1919 should be conserved?

The Editorial Board (1954) published a preliminary statement on the status of the generic names, Escherichia, Klebsiella, Aërobacter and Cloaca. The Enterobacteriaceae Subcommittee (1954, 1958) reported on these genera. In the 1958 Report, the Subcommittee proposed to define Klebsiella as consisting of nonmotile, encapsulated bacteria that conform to the definition of the family Enterobacteriaceae and to define Cloaca (Aërobacter) as consisting of motile bacteria that conform to the definition of the family Enterobac-

teriaceae, and give typical biochemical reactions. The Subcommittee pointed out that cultures of Klebsiella which have been typed were derived largely from respiratory infections and urinary infections in man and from human feces.

Winslow et al. (1919), Weldin (1927), Ruchhoft et al. (1931), Ostermann et al. (1941), Olsen (1942), and Borman et al. (1944) all noted the difficulties experienced by workers in trying to classify the Klebsiella-Aërobacter group on biochemical tests, particularly sugar fermentations. Ostermann et al. state that they found no single test or group of tests that would distinguish many of their Klebsiella (Friedländer) cultures from those of the coli-aërogenes group and state that it seems not unlikely that much of the confusion still surrounding the Klebsiella (Friedländer) and coli-aërogenes organisms is due to failure to recognize the full significance of variation.

Edwards (1929) isolated strains of Aërobacter from feces, soil, water, and milk and noted that both the Aërobacter strains and the Friedländer (Klebsiella) strains were rather variable in their fermentative characters.

Parr (1938) noted the difficulty of classifying the coliform bacteria owing to the essentially intergrading nature of the organisms in this group and defined intermediates as coliform organisms which have one or more of the characteristics of Escherichia coli and one or more of those attributed to Aërobacter aërogenes and notes "among the coliform bacteria characters are from time to time lost or those in abeyance are regained." Parr (1939) noted that these intermediate forms either do not conform to the so-called LMViC pattern, or lack power to ferment certain carbohydrates, or produce only acid where the production of acid and gas is considered normal, or display a combination of fermentation characters the reverse of that which normally occurs. It is to be noted that Parr (1936) isolated strains of Aërobacter from feces which had been stored in an ice box for as long as two months.

Stuart et al. (1938) noted that, with some of their 5,200 cultures isolated from human and bovine feces, from dust of cowbarns, and from milk, growth was more luxuriant at room temperature than at 37°C and that cultures (grown in methyl red medium) that produced only acid when grown at 37°C for 36 hours produced acid and gas when grown at room temperature for the same period, and also that the methyl

red reaction varied with temperature of incubation. These irregularities in the methyl red reaction were most noticeable in the strains which either failed to produce gas from lactose at 37°C or produced gas at room temperature only.

Stuart et al. (1940) proposed the term "aberrant" coliforms for cultures not fermenting lactose, producing acid only, or requiring more than 48 hours for the production of gas at 37°C. These strains were isolated from water, soils and cereals. Many of the so-called "aberrant" strains grew abundantly at room temperature but sparsely or not at all when incubated at 37°C (in other words they isolated mesophilic strains of Aërobacter).

The Enterobacteriaceae Subcommittee (1958) first gives the biochemical characteristics of the Cloaca (Aërobacter) group and then states "Although these biochemical reactions can be considered as typical of the group, there are aberrant cultures, not fermenting lactose, sucrose, rhamnose, sorbitol, raffinose, methyl red positive, Voges-Proskauer, citrate, or KCN negative. Anaerogenic strains also exist."

My work on the coliform group as a whole has convinced me that a satisfactory classification of the Klebsiella-Aërobacter group cannot be based solely on sugar fermentations. That this fact is generally recognized is shown by the many qualifications found in the literature such as "frequently do not attack"; "may or may not attack"; "many strains ferment"; "usually do not form acid and/or gas." Using a pH meter one can state that fermentation has occurred, but cannot state that a coliform culture is not capable of developing the ability to ferment a given carbohydrate. In carrying out the methyl red and Voges-Proskauer reactions Fouad et al. (1953) have shown the necessity for a chemically defined glucose ammonium phosphate medium.

Some strains of Aërobacter do not produce gas in lactose broth at 37°C but do so at 15-30°C. Begue and Lichstein (1958) have shown that in Saccharomyces cereviseae the required synthesis of pantothenic acid is accomplished at 30°C but in some manner is prevented at 37°C, i.e. an enzyme-temperature system is postulated. Lichstein (1960) notes "it is becoming increasingly apparent that antagonisms and interactions among nutrients as well as the character of the physical environment influence markedly the nutritional demands of an organism" and "genetic expression is affected by the environment and the presence of a particular gene

does not necessarily mean that the corresponding character will be expressed."

The ability of non-lactose-fermenting organisms isolated from feces to ferment lactose after long cultivation in lactose media has been shown by a number of workers, particularly Kriebel (1934) who draws the conclusion that these strains are closely related to the colon group, possibly as variants, since they tend to dissociate into lactose-fermenting coli-like organisms. Tregoning and Poe (1937) found that the production of sucrose-positive variants was readily accomplished in some strains of Escherichia and Aërobacter. Di-Girolamo et al. (1958) have shown that strains of E. coli that did not use arabinose as the sole carbon source did so if seeded on a mixture of rhamnose and arabonise, and noted that the methylpentose is an indicator of the enzyme of arabinose metabolism, which by itself has no inductive property.

Breed (1957) in Bergey's Manual comments on the unsatisfactory position of the Klebsiella-Aërobacter group and notes that no method has been found to differentiate the majority of K. pneumoniae strains from urinary strains commonly classified as A. aërogenes. He concludes that while awaiting a better solution of the problem it was felt advisable to continue to recognize A. aërogenes (as a species distinct from K. pneumoniae) and both genera Aërobacter Beijerinck and Klebsiella. He pointed out that species of Aërobacter that occur in dairy products are frequently derived from grain and that they are found on panicles of the grass family in open fields.

The Enterobacteriaceae Subcommittee (1958) recommended biochemical methods for differentiation of the genera of the Enterobacteriaceae. Except for gelatin liquefaction at 21°C, they suggested that tests be carried out at 37°C for 2 days. If negative, follow with further incubation at 21 to 25°C for 5 days. In our work on the cold-tolerant mesophilic strains of Aërobacter, the above methods have been followed since 1928 at an incubation temperature of 21°C, since these strains grow poorly or not at all at 37°C. It is difficult to compare our data with those of previous workers before 1950, since others usually worked with nonmotile cultures and carried out their tests at 37°C. The methyl-red test particularly is influenced by the temperature of incubation, since temperature influences the rate of production of acid in the medium.

Schafler et al. (1959, 1960) obtained through "training" on lactose media and by selection lactose-positive strains of Salmonella which also became cellobiose-positive. An interesting finding was that the appearance of lactose-positive variants from cellobiose-positive variants of Salmonellae was particularly or totally inhibited by succinate or glutamate. Schema et al. (1943) found increasing tolerance of A. aërogenes to sodium pentachlorpentate and noted that in certain cultures gas was no longer formed from arabinose or rhamnose, and that in such cultures the methyl-red test became positive.

That temperature of chemicals can cause gene unstabilization has been shown by Zamenhof and co-workers (1958, 1961). They used a typical strain of E. coli and a lactose nonfermenting mutant of that strain. A similar situation may well apply to strains of Klebsiella and Aërobacter. There is also the question of growth requirements and I suggest that mesophilic (plant) strains of Aërobacter ingested in the food of an animal (given time) would adapt themselves to their habitat and that the ability to ferment lactose or other properties could be lost. Such a strain later isolated from the urinary tract or from feces might well be identified as Klebsiella or as an intermediate strain. Martinec et al. (1961) discussing the taxonomic status of Serratia marcescens Bizio state that relatively great variability of some strains were observed during the fermentation of carbohydrates and add "it is interesting that the majority of these anomalies was observed in strains isolated from different species of insects."

As a dairy bacteriologist, I have been interested since 1925 in strains of Aërobacter that fermented lactose poorly or not at all at 37°C. They were first isolated from creamery water supplies contaminated by soil. Since in a creamery water supply it is as important to eliminate soil contamination as to detect fecal contamination, in routine examination for the coliform group lactose-broth tubes were inoculated in duplicate at 21°C and at 37°C. This procedure, also applied to milk and milk products, led to the isolation from water, milk, cream, poor quality butter, gassy cheese, and ice cream, of strains of Aërobacter whose optimum temperature ranged from 20°C to 30°C, that grew well at 15°C and grew slowly at 4°C (1931).

Grimes (1934) concluded that while it is now usual to

carry out the test for Escherichia and Aërobacter from water and dairy products at 37°C the test should also be carried out also at 21°C, otherwise the Aërobacter group, although present, may not be identified. These coli-tolerant mesophilic strains of Aërobacter have been isolated by many workers from soil, grasses, ears and panicles of cereal crops, milk, cream, poor quality butter, gassy cheese, ice cream, creamery and farm water supplies and dairy farm equipment, chilled meat and crabmeat.

A characteristic of these strains of Aërobacter is their poor growth or lack of growth in lactose broth at 37°C. Thomas and co-workers (1958) state that out of 1040 cultures isolated from raw milk and farm dairy equipment which fermented lactose with the formation of acid and gas to 30°C, 473 (nearly 50%) failed to produce gas from lactose in two days at 37°C.

Although this paper is concerned mainly with mesophilic strains of Aërobacter I have also worked on the strains that grow best at 37°C, likewise strains of Escherichia and various intermediate strains. I am of the opinion that while one can state that a particular strain is capable of a particular fermentation, it cannot be said that it is incapable of fermenting that substance. Whether or not fermentation occurs depends on the original environment, medium used, temperature of incubation and length of time of incubation.

The growth medium and the temperature of incubation may also influence motility. I find peptone broth the best medium for determining motility, the culture to be incubated for 24 hours at its optimum temperature. One does not get as good results with lactose broth, for the strains of Aërobacter grow plumper and larger, and in the motility test they are found to be sluggishly motile or nonmotile.

I am in agreement with Hormaeche and Edwards (1960) that capsules and slime formation are of no value in differentiation since the related strains are not constant in these properties. In our routine work (incubation temperature 21°C), streaking or plating on Levine media from lactose broth cultures showing acid and gas at 21°C in 24 to 48 hours the mucoid strains are readily separated from the nonmucoid strains, but I have not found it possible definitely to separate them by biochemical tests. The mucoid strains will cause ropiness in milk and give sliminess in glucose, lactose, and sucrose broth and more or less slimi-

ness in galactose, raffinose, sorbitol, xylose and glucerol broth. There is no correlation between mucoid growth or nonmucoid growth and the liquefaction of gelatin.

In classifying Klebsiellae and Aërobacteres one is between Scylla and Charybdis, since on the one hand it is possible to simplify by restricting the number of tests, e.g. relying on IMViC and the fermentation of lactose and glucose, or on the other hand to complicate to the point of absurdity by differentiating on numerous fermentation and other tests. There is the further complication that the influence of medium, growth temperature and length of time of incubation has not always been fully recognized. Alford (1960) and Schultze et al. (1960) support our contention of the importance of incubation temperature when carrying out biochemical tests. Taylor's question (1959) "Why Christen a Salmonella?" is pertinent here, and the same question can be asked also regarding the Klebsiella-Aërobacter group. The cold-tolerant mesophilic strains of Aërobacter especially are to be regarded as an adaptable group capable of changing to meet changes in their environment (given time) whether it be biological or biochemical.

In discussing the legitimacy of the generic name Klebsiella to include both Klebsiella and Aërobacter, the report of the Coli-Aerogenes (1956) Subcommittee of the Society for Applied Bacteriology (1956) states "since the generic name Klebsiella has priority over Aërobacter, the aerogenes organism not only falls into the genus Klebsiella but, strictly, also loses its specific name. However, if the Rules of the Bacteriological Code (1948) are strictly applied, the correct names for many coli-aerogenes bacteria would be so unfamiliar as to cause confusion and error, and it will be necessary to ask for official opinions to conserve suitable names against earlier. The views experssed by Cowan (1954) concerning names for coliform organisms and his paper "Nonconformism in Nomenclature" (1959) are also relevant.

Hormaeche and Edwards (1960) have discussed the difficulty of classifying the bacteria of the Klebsiella-Aërobacter group. They proposed a new genus Enterobacter with the type species Enterobacter cloacae based on Bacillus cloacae Jordan (1890) and requested that the term Enterobacter Hormaeche and Edwards be placed in the list of conserved bacterial generic names and that Enterobacter cloacae Jor-

dan comb. nov. (B. cloacae Jordan, 1890) be designated as its type species. This request of Hormaeche and Edwards deserves careful consideration, but it accepts in general only those strains of Aërobacter with an optimum temperature 37°C, and rejects the cold-tolerant mesophilic strains of Aërobacter of Grimes and Hennerty (1931). Ewing and Edwards (1960) state "that at present the Aërobacter group is divisible into three subgroups corresponding to the species A. cloacae, A. aërogenes and A. liquefaciens Grimes (1931)."

Hormaeche and Edwards (1958) make a valuable contribution in their observations on the genus Aërobacter with respect to the separation of Aërobacter from Klebsiella on the basis of motility and the urease and ornithine decarboxylase tests. They note that nonmotile variants of otherwise typical cultures are known to occur, likewise aberrant and intermediate strains. They state that there are at least two well-defined species—A. aërogenes and A. cloacae—and list their biochemical characteristics. I am of the opinion that sanitary and food bacteriologists would be in agreement in desiring that the generic name Aërobacter be retained even though Klebsiella has precedence. Classification is not intended to fit a concept of static genera and species. With the development of more and better taxonomic methods, particularly serological methods, the interrelationships of the strains that comprise the Klebsiella-Aërobacter group are now somewhat better realized. Can Klebsiella be differentiated from Aërobacter while acknowledging the adaptability of the latter group to adjust itsel to its environment, resulting in a variety of intermediate strains, which may also be termed "variant" or "aberrant"?

The description of the genus Klebsiella, as given by the Enterobacteriaceae Subcommittee (1958) and in Bergey's Manual (1957), I would amend as follows: Nonmotile short rods, Gram-negative, encapsulated in the mucoid phase, conforming to the definition of the family Enterobacteriaceae, methyl-red negative, Voges-Proskauer positive, gelatin not liquefied, citrates utilized as sole source of carbon, nitrites produced from nitrates, sodium malonate positive, capable of fermenting glucose, lactose, sucrose, mannitol, salicin, and growing in KCN medium. The type species is Klebsiella pneumoniae. Encountered frequently in the respiratory, intestinal and urogenital tracts of man and recognized

as one of the causes of mastitis in cattle (Barnes, 1953; Buntain and Field, 1953; Esterbrooks and Plastridge, 1956; Guillot et al. 1961; Smith and Henderson, 1934; White, 1957; The type species represents the strains of the group that are adjusted to a human or animal habitat, and may be more or less pathogenic.

As to the value of biochemical tests in distinguishing A. cloacae from A. aërogenes, the Enterobacteriaceae Subcommittee (1958) give the biochemical characteristics of Cloaca (Aërobacter) and state "although these biochemical characteristics can be considered as typical, there are aberrant cultures not fermenting lactose, sucrose, rhamnose, sorbitol, raffinose, methyl-red positive, Voges-Proskauer, citrate or KCN negative, Two biochemical types can be differentiated. Type A does not generally ferment inositol nor glycerol, and when doing so does not produce gas in four days; it is arginine positive, lysine negative and liquefies gelatin. Type B promptly ferments with gas inositol and glycerol, is arginine negative, lysine positive and about hals the strains do not liquefy gelatin."

Breed (1957) states "usually motile" gelatin colonies—"thin, circular, bluish, translucent" (no mention of liquefaction). Gelatin stab—"slow liquefaction, liquefying power sometimes lost."

I regard a nonmotile A. cloacae strain as a variant of A. aërogenes Beijerinck, 1900, i.e. a Klebsiella strain; a motile A. cloacae that does not liquefy gelatin as not properly identified and the motile A. cloacae strain that liquefies gelatin, more or less slowly, as a variant of A. lipolyticus nom. nov. described below.

Aërobacter is defined as follows: motile, Gram-negative rods, mucoid phase common in media containing lactose, conforming to the definition of the family Enterobacteriaceae, methyl-red negative, Voges-Proskauer positive, uric acid, citric acid and citrates utilized as sole source of carbon, nitrites formed from nitrates, fermenting with the production of acid and gas—glucose, levulose, galactose, lactose, sucrose, maltose, cellobiose, mannitol, raffino e, sorbitol, inositol, rhamnose, arabinose, xylose, glycerol, salicin, aesculin, α-methyl glucoside; positive for arginine dihydrolase, lycine and ornithine decarboxylases; sodium formate and sodium malonate positive. Growth in KCN medium. Phenylalanine deaminase negative. Dulcitol,

inulin, potato starch, destrin may or may not be fermented. Indole test usually negative.

These biochemical reactions are typical for the strains that comprise the cold-tolerant mesophilic strains, optimum temperature, 20°C to 30°C; good growth at 15°C. growth at 4°C; poor or no growth at 37°C.

Soil and plant strains, isolated from soil, grasses, cereals, water, milk and milk products, and cold-stored meat. Cause of ropy milk. Aërobacter lipolyticus nom. nov. liquefies gelatin. Pectin not liquefied.

A strain with the same biochemical characteristics, but incapable of liquefying gelatin, has been named Aërobacter hibernicum.

The name Aërobacter liquefaciens Grimes and Hennerty 1931 is a later homonym of Aërobacter liquefaciens Beijerinck 1900, hence illegitimate. The name Aërobacter lipolyticus nom. nov. is proposed to replace A. liquefaciens Grimes and Hennerty. A detailed description is given herewith.

Aërobacter lipolyticus nom. nov.

Synonym: Aërobacter liquefaciens Grimes and Hennerty 1931, not A. liquefaciens Beijerinck 1900.

Morphology: Rods, 0.75-1.0 x 1.0-2μ, motile, possessing pertrichous flagella, Gram-negative, aerobic, facultative, nonspore-forming.

Nutrient gelatin: Two days at 21°C. Punctiform, entire dirty greyish colonies, gelatin liquefied.

Nutrient gelatin stab: Two days at 21°C. Filiform growth with liquefaction. Five to seven days—above 10% liquefaction, infundibuliform. Two to three weeks—around 40% liquefaction.

Tryptone dextrose agar: Two days—colonies, circular, smooth, moist, glistening, greyish-white, viscid, convex, opaque, raised, entire.

Tryptone dextrose agar slant: Two days—abundant greyish-white growth, smooth, moist, glistening, opaque, viscid.

Nutrient broth: Good growth in 24 hours, turbid.

Levine medium: One to two days—good, nonchromogenic growth. Colonies or streak-smooth, glistening, opaque, viscid.

Potato slope: Two to five days—good growth, smooth, moist, glistening, opaque, viscid, muddy grey to brownish grey colour.

Biochemical Tests at 20-30°C

Methyl-red: Negative.
Voges-Proskauer test: Positive.
Uric acid media: Two days—good growth. pH 4.4-5.0.
Koser's citrate medium: Two days—good growth.
Hydrogen sulfide: Lead acetate filter paper strip—Negative.
Bismuth liquor technique. 1938. J. Bact. 35:183. Definite blackening around the top of the medium and along the line of the stab.
Nitrates reduced to nitrites.
Indol: Usually negative (positive strains found).
Catalase: Positive.
Diastatic action: Negative.
Milk: Acid coagulation, ropiness, pH 5.3 in two to three days. Acidity 0.5 to 0.6% lactic acid.
The following are fermented with production of acid and gas, with pH dropping to 5.0 or lower in two to four days: Lactose, sucrose, fructose, galactose, maltose, rhamnose, arabinose, cellobiose, aesculin.
pH dropping to less than 5.5 but not lower than 5.1: Mannitol, inositol, adonitol, xylose, raffinose, α-methyl glucoside, salicin.
pH dropping to 6.6 but not lower than 5.6: Sorbitol, glycerol, amygdalin.
Glucose: Drops to pH 5.5 in two days and is around 7.0 in four days.
Dulcitol may or may not be fermented; when fermented, pH varied from 5.5 to 6.5.
Erythritol: Not fermented.
Mucoid phase common in media containing glucose and lactose.
Inulin: Usually fermented, pH 6.3 (average).
Dextrin: May or may not be fermented; when fermented, pH 6.4 (average).
Starch: May or may not be fermented; when fermented, pH 6.0 (average).
Sodium malonate: Positive, pH 8.4 (average). One negative strain found.

Sodium hippurate: May be negative or positive, pH 6.2 (average).
Sodium formate: Positive, gas production, pH 8.4 (average).
KCN: Positive.
Asparagine: Positive, pH 8.0 (average).
Arginine dihydrolase: Usually positive.
Lysine decarboxylase: Positive.
Phenylalanine deaminase: Negative.
Ornithine decarboxylase: Positive
Growth in 7.5% sodium chloride solution.

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A NEW SALMONELLA TYPE: S. LAWNDALE

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SUMMARY: A new Salmonella serotype, (9, 12: z-1, 5) S. lawndale, is described. It is indol positive and was isolated from a case of enteric infection in a human.

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The new serotype to be described was received for confirmation from the Illinois Department of Public Health. It was isolated by Dr. William I. Metzger at Cook County Hospital, Chicago, from the stool of a three-month-old negro female with acute gastroenteritis.

The culture produced indol, was methyl-red positive, Voges-Proskauer negative, grew readily in Simmons' citrate medium, and produced hydrogen sulfide, but failed to hydrolyze urea or to liquefy gelatin. The lysine and ornithine decarboxylase tests and the arginine dihydrolase test were positive. When tested by the method of Kauffmann and Petersen (1956), D-tartrate and L-tartrate were utilized properly but I-tartrate, mucate and citrate were not attacked. Malonate was not utilized. The culture promptly produced acid and gas from glucose, arabinose, rhamnose, xylose, maltose, sorbitol, trehalose, mannitol and dulcitol and acid from glycerol; acid was produced from cellobiose after 4 days incubation. Lactose, sucrose, salicin, adonitol, inositol, and raffinose were not attacked.

S. lawndale was a member of Salmonella group D and in absorption tests removed all agglutinins from Salmonella gallinarum O (9, 12) serum. The H antigens were diphasic and phase 1 agglutinated to the titer of Salmonella poona phase 1 (z) serum and in absorption tests removed all agglutinins from the serum. Phase 2 was agglutinated by Salmonella thompson phase 2 (1, 5) serum. It was readily agglutinated by single factor 5 serum but not by sera for factors

2, 6, 7 and z_6. In absorption tests it reduced the titer of S. thompson phase 2 serum from 1-10,000 to 1-100. S. lawndale was assigned the antigenic formula 9, 12: z-1, 5.

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ON EXTERNAL FEATURES IN THE TAXONOMY OF ACTINOMYCETES

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SUMMARY: The usefulness of several characters in the differentiation of taxa included in the Actinomycetales is reviewed. Groups (taxa) are defined in each case not by a single character but by a combination of several. The colourless actinomycetes with white aerial mycelium include one group with straight sporophores and one with spiral. In green, violet and blue groups the solubility of pigments produced and the nature of the fructification are important. If the groups are established according to external morphological and cultural characters, then the species included in the groups are differentiated according to the physiological, biochemical and antimicrobial characters and the specificity of mutual antagonisms. Within the same group the species differ in the complex of characters. In the globisporus group 8 species are recognized, 7 species and 2 subspecies in the violet group, in the bluish white group, 2 species, in the brown-green group 2 species and in the fluorescent group 3 species.

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The problems of taxonomy, the extablishment and identification of species of actinomycetes are currently regarded as most important and urgen in antibiotic science.

We have repeatedly noted that the confusion existing in this field greatly impedes the work on finding and studying antibiotics. This circumstance has caused the International Taxonomy Committee to set up a species Subcommittee on Taxonomy of Actinomycetes.

At the first sitting of this Subcommittee, which was held in Stockholm in August 1958, it was decided to carry out an international study of a specially selected collection of 25 actinomycetes. Thirty-two laboratories in various countries —Germany, Italy, Japan, USA, USSR and others—have taken part in the processing of these cultures.

The cultures were studied according to an established scheme and program by identical methods using identical nutrient media. The study continued for about two years. The results of the study were collected by the Subcommittee, (Secretary Dr. Küster and others) and duly processed. A brief summary of this processing of the experimental material has been circulated among the participants.

The results obtained are far from uniform. The same features in the same strains are represented differently. Even morphological indications such as the shape and the structure of sporophores and spores yield widely divergent data. For instance, of 87 analyses of strain No. 1 (made in 32 laboratories), 17 show straight sporophores and 70 spiral ones; of 88 analyses of strain No. 2, 77 show straight sporophores and 11 spiral. Similar data were obtained for the remaining cultures (Table 1).

As to spores, the results are more definite and in better agreement. Here too, however, some divergency is observed. For instance, in 32 analyses of strain No. 1 the spore membrane is given as smooth, while in one case it is prickly; in 3 analyses of strain No. 12 the spore surface is defined as smooth, in 28 analyses as spiny; strain No. 21 in three cases had smooth spores, in 27 cases spiny, and in one case hairy. In all, such discrepancies in respect to spores amount to 11 out of 32 analyses.

There is a divergence of opinion and results in establishing the mode of branching of mycelium in the tested 25 cultures of actinomycetes. The same strains exhibit either only monopodial branching or monopodial and verticillate, the verticils being both primary and secondary. For instance, 8 analyses of strain No. 1 yield primary verticils with spiral sporophores, while one analysis gives secondary verticils and straight sporophores. Similar results were obtained for strains Nos. 8, 21. Strains 2, 3, 6, 7, 10 in the majority of cases exhibit monopodial structure of sporophores, though there are individual cases of verticillate structure (Table 2).

Table 1. Structure of sporophores and spores according to data from various laboratories of the world. Number of strains with a given character.

Actino-mycetes	Sporophores		Spores		
	Spiral	Nonspiral	Smooth	Spiny	Hairy
Strain 1	70	17	32	1	0
" 2	11	77	31	1	0
" 3	7	79	33	0	0
" 4	89	9	32	0	0
" 5	82	8	31	1	0
" 6	12	81	34	0	0
" 7	89	25	26	0	1
" 8	77	22	33	0	0
" 9	94	28	34	0	0
" 10	56	36	32	0	0
" 11	79	18	29	0	0
" 12	77	17	30	0	0
" 13	72	7	3	28	0
" 14	1	81	28	3	0
" 15	2	59	22	2	0
" 16	40	25	24	0	0
" 17	0	79	33	0	0
" 18	1	87	32	1	0
" 19	76	14	3	29	0
" 20	78	10	0	28	1
" 21	73	6	3	27	1
" 22	0	27	8	0	0
" 23	70	15	23	1	0
" 24	1	52	20	0	0
" 25	76	11	27	0	0

Table 2. Character of sporophore—branching according to data of various laboratories resulting from analysis of international collection of actinomycetes.

Strain Nos.	Total number of analyses	Established as verticillate			
		With primary verticils		With secondary verticils	
		Spiral	Straight	Spiral	Straight
1	87	9	0	0	1
2	88	0	2	0	0
3	87	0	2	0	0
5	90	0	0	3	0
6	93	0	1	0	0
7	113	0	3	0	0
8	99	7	0	2	0
10	89	0	3	0	0
13	90	3	0	0	0
19	90	2	0	0	0
20	88	19	0	0	3
21	79	13	0	5	0

Such contradictory data give an impression that the characters given cannot differentiate the species and hence are generally unfit for use in taxonomy.

No less inconsistent are the data on the suitability of the external features for the determination of actinomycetes species, found in the works of various authors. This is also evident in papers published in the journal "Microbiologia" as part of discussion on this subject (Waksman, 1959; Tesic, 1960; Baldacci, 1959; Kosmachev, 1959; and others).

We have made a thorough study of the collection of strains circulated by the Subcommittee. Each strain was studied comprehensively: morphologically, culturally, physiologically and biochemically. Each culture was investigated from the standpoint of its antimicrobial and antagonistic features, etc.

Based on our own experience in general and on the results obtained in processing the above-mentioned collection, we shall attempt to systematize the material collected by the

Subcommittee and to present our views and explanations concerning the puzzling contradictions and inconsistencies.

In the first place, it is useful to touch upon the question of understanding and representing the characters. What is the taxonomic significance of individual characters?

The scheme suggested by the Subcommittee is based on the subdivisions of the shape of sporophores after Pridham et al., (1958). According to this scheme, the nonspiral sporophores are divided into two types: straight and flexuous, single and fascicled. The spiral sporophores are subdivided into, a) typical spirals with many coils which are stretched or tightly compressed; b) primitive spirals in which the sporophores terminate in an incomplete spiral coil of 0.5-1 turn; c) spirals formed by the vegetative filaments of a mycelium, etc.

Actinomycetes having the above-mentioned sporophore structure are divided into sections (Spira, Reticulum-apertum).

According to our data such a subdivision has no sufficient experimental basis. One and the same species with nonspiral sporophores may have straight and flexuous, short and long sporogenous branches. Our collection includes more than 350 strains of the same species (Actinomyces streptomycini), a streptomycine producer, collected by us from various soils of the USSR. They are all similar in their physiological, biochemical and antimicrobial properties. Their sporophores are nonspiral, but with some strains they are short and straight, with others long and flexuous; occasional sporophores are curved (but not spiral), they bear a slight resemblance to irregular spirals.

The same strain may have long flexuous or short straight sporophores depending on the conditions of growth and the composition of the nutrient medium.

Similar features are exhibited by other species: A. olivaceus, A. viridis, etc.

We believe that nonspiral sporophores are all of the "nonspiral" type, no matter whether they are short and straight, long and flexuous, or curved.

There may occur individual species with short, bristly, straight sporophores, sufficiently characteristic and stable, hereditarily fixed. In such cases the given character is correlated with other characters: physiological and biochemical.

The concept of spiral sporophores is often no longer justified and quite arbitrary. They are either excessively differentiated or insufficiently analyzed, which results in erroneous determination.

Let us consider the so-called "primitive sporophore," i.e. a sporophore with an incomplete terminal coil (0.5-1 turn). According to our observations such sporophores are found consistently only in individual actinomycetes. In such cases this character is always correlated by other characters, and we are inclined to regard it as specific, inherent in definite organisms. As a rule, they ha e no real spiral sporophores with many coils. However, we class such sporophores as spiral.

In the majority of actinomycetes such incomplete coils of spiral sporophores are unstable and occur only occasionally, depending on the conditions of growth, the composition of the medium and other factors. They are often formed in the same culture along with complete and numerous coils. We consider such cultures as having fully formed spiral sporophores. "Imperfect" spirals are not typical of them, and represent a manifestation of polymorphism, not a specific character.

Some actinomycetes form highly characteristic and tightly compressed spirals appearing as a glomerule or a miniature fist on the end of the sporophore. So far we are unable to say to what extent it is a specific characteristic and what its hereditary stability is, since no special investigations have been carried out. We may note, however, that in many cases cultures with such sporophores possess also a number of other distinguishing features of correlative nature. At the same time many strains of actinomycetes having sporophores tightly compressed into fists, do not differ in any respect from strains with conventional spiral spore chains.

In our classification of actinomycetes we regard the above-mentioned curls in the glomerules as spiral formation of sporophores.

Of very vague nature is the spiral coiling of filaments of the vegetative mycelium. It is common knowledge that in cultures of actinomycetes one may observe occasional formation of long "corkscrew"-curling of mycelial hyphae at various sites along its length. Such filaments are not associated either with sporogeny or with any other phase of the culture development. No spores or any other reproducing

elements are found in spirally coiled filaments.

It should be noted that such mycelium curls are observed in mycelial fungi, and sometimes in filiform bacteria. The nature and cause of their appearance are yet to be learned. Our observations show that this character in actinomycetes is not stable, it far from consistently manifests itself in the same strain, and no relation between it and the conditions of the culture growth can be detected. There are no grounds as yet to accept it as a taxonomic character in determining and differentiating species.

It is noteworthy that actinomycetes having spiral hyphae in the vegetative mycelium, generally form spiral sporophores. The latter may be rare and may remain unnoticed in a cursory examination of the culture.

In actinomycetes with typical spiral sporophores spirals are not always (and not by everyone) detected in the same culture. For instance, 92 analyses of strain No. 10 showed 56 spiral and 36 nonspiral sporophores. Eighty-eight analyses of strain No. 20 are said to have yielded 78 spiral and 10 nonspiral sporophores. Similar contradictory data were obtained with other strains investigated (Table 1).

What is the cause of such a contradiction? How shall we regard actinomycetes with such nonuniform characters: shall we class them as spiral or nonspiral cultures?

We believe that if any strain has even a single spiral sporophore on any medium, it should be classed as a spiral culture. It is well known in mycology that very many species form ascospores quite rarely, they usually multiply by means of a vegetative reproduction; many investigators are unable to detect asci in them; nevertheless such species are classed as ascomycetes. As an illustration, we can quote representatives of the species Aspergillus, Penicillium, etc. In brewer's yeast, spores are detected very rarely, the cells multiply by budding as is usual in asporogenous yeast, and still this yeast is classed as sporogenous and included in the genus Saccharomyces.

Many similar examples could be cited in mycology where the true place of the culture in the system of organisms is established by a single detected sporophore. Similar cases are found in bacteriology. Certain strains of sporogenous bacteria form single spores, and only in strictly definite media and under definite conditions of growth. For instance, some cultures of sporogenous oligonitrophils form spores

very rarely; to find cells with spores requires careful microscopic study of the preparations, while in many preparations no spores whatever can be detected. Nevertheless, we class such cultures among definite species of sporogenous bacteria, and not among the group of nonsporogenous forms.

The actinomycetes should be no exception.

Based on our long experience we can state that if this or that strain shows even individual spiral sporophores only in certain media or in a single medium, it is possible to achieve a more abundant spiral formation in the future.

We have noted repeatedly that the detection of spirals, like that of any other character, requires definite conditions of nutrition and growth.

Routine laboratory practice frequently reveals, in the same strain, the presence of spirals in some cases and their absence in others; the spirals are more abundant in some media, while others do not exhibit them at all or show them in rare, isolated cases. Some strains form spiral sporophores only in definite media. Sometimes we have to test many nutrient media, change individual medium components, try separate sources of carbon and nitrogen, very the pH of the medium or the intensity of aeration, the temperature of growth, etc.

There is no single medium for spore formation which would be optimal for all species of actinomycetes. Although most cultures give the highest yield of spiral sporophores on deficient or semideficient synthetic media, on the Czapek medium, SM-1 etc., one should not restrict himself to these in spiral detection. Different media should be tried to make sure that the culture under study can be classed among actinomycetes with nonspiral sporophores.

Some strains form spiral sporophores on a synthetic medium with glucose and maltose; others, with sucrose or mannitol, still others, with glycerol or starch or some other carbon nutrient. Different behavior with respect to nitrogen nutrition is observed. Some strains form spirals preferably on synthetic media with nitrate nitrogen, others, with ammonia nitrogen.

Many or most actinomycetes have abundant spiral sporophores on deficient (water-agar) or semideficient (SM-1 or Czapek, Ashby-agar with 0.2% carbohydrate) media. Cultures grow very slowly on these media. Often their

growth cannot be discerned by the unaided eye. There is growth, however; using a microscope, one can see the development of mycelium filaments creeping over the surface of the agar, and numerous sporophores branching off from the mycelium.

The process of sporogeny and the sporophore formation is greatly affected by the composition of the mineral salts used for the media. Even tap water may influence this process. It is well known that the composition of tap water varies markedly depending on the season. In spring it is not the same as in summer or in winter. It is also different from reservoir to reservoir. The water composition of mountain rivers differs from that of rivers flowing through lowlands and particularly through swampy areas.

Very substantial influence is exerted on sporogeny by trace elements contained in nutrients.

The optimal temperature for sporophore and spore formation in actinomycetes lies between 20 and 30°C (it is considerably higher for thermophils). At 37°C sporogeny is slow, while many species do not exhibit it at all, the colonies remain bare or reveal sterile aerial mycelium. Abundant formation of spores and spiral sporophores is observed at 15-20°C, but it is slower than at 25-30°C (Krasil'nikov, 1938).

Some actinomycetes react to the composition of the glass vessel in which they are growing. New or poorly washed (unleached) test tubes or retorts may produce marked changes in the character of sporogeny. It is not a rare occasion that two closely spaced test tubes containing identical cultures planted at the same time show an abundance of sporophores in one test tube and little or none in the other.

In our earlier investigations we showed (1938-59) that cultures for which spiral sporophores are not typical, never form them, and vice versa. This may be illustrated by strains of the globisporine group which have been thoroughly studied by us.

Our laboratory surveyed more than a thousand cultures of this group of actinomycetes. They have been classed into 8 species, all having nonspiral sporophores. Six hundred fifty strains belonging to these 8 species have been subjected to special investigation. In these we have never observed spiral sporophores or sporophores with even 0.5-1

coil. We grew them in various media: in deficient or semi-deficient synthetic media or in rich complex, albuminous media, in natural vegetative substrates (carrots, potatoes, etc.); in all cases the cultures had typical nonspiral sporophores.

We have reported earlier (1958) that these cultures were subjected to experimental mutability. Under the effect of various mutagenic influences and spontaneously, many variants were obtained which differed from the original and among themselves in physiological and cultural characters; there were variants with a different nature of colony growth, with another shade in the colouring of the aerial mycelium; we had strains which were entirely devoid of aerial mycelium, and also variants differing in the assimilation of carbohydrates, in enzymatic and other properties. However, we have never noted cultures which changed the shape of the sporophores, i.e. turned from nonspiral to spiral cultures.

We carried out similar investigations of experimental mutability with certain cultures forming typical spiral sporophores—A. violaceus, A. coelicolor. These organisms form numerous and well-pronounced spiral sporophores with 3-6 coils. We tried to make them produce mutants with straight sporophores. In the process of experimental variability from violet actinomycetes we have obtained more than 50 mutants: some of them are colourless, others are coloured definitely red (not violet), forming a single red or pink pigment; there were mutants without aerial mycelium with typical black-violet bare colonies, also mutants which did not colour the medium, i.e. those forming chromophorous pigments (which are not excreted into the medium). Many mutants featured varying intensity of proteolytic, amylolytic and other fermentation activity. We had strains with rare spiral sporophores which, in addition to that, formed them by no means in all media. However, we did not obtain any variant entirely lacking in ability to form spiral sporophores.

A blue actinomycete, A. coelicolor, yielded about 250 variants: pigmented and colourless, with and without aerial mycelium, variants with different colour shades, chromoparous and chromophorous; many variants had biochemical and enzymatic distinctions. But there was not a single strain which would change the type of sporogeny and acquire typical nonspiral sporophores. Similarly to the preceding

cases, there were strains with rare spiral sporophores formed only in definite media. However, they possessed typical spirals.

The foregoing shows that the shape of sporophores in actinomycetes is a sufficiently stable, hereditarily fixed and always clearly pronounced character under proper conditions. Consequently, this character is also most suitable in taxonomy when establishing species, groups or subgroups.

In our opinion, the data presented here explain the contradictory results obtained by various investigators in the study of the international actinomycete collection. In all cases where obviously spiral actinomycetes did not exhibit spiral sporophores, the strains were not studied thoroughly enough. In some cases the variability and polymorphism of cultures were not taken into account. This is confirmed by our investigations.

Considering such a great divergency of results of the above-mentioned investigators, we placed particular emphasis on studying the same actinomycete collection. At first we strictly adhered to all the methods of culture studies recommended by the Subcommittee. We used the media and the conditions of growth recommended in the instructions.

We were not satisfied with results obtained and worked out an extended program of investigations, particularly with regard to those strains that yielded dubious results. For spore formation and culture growth we used synthetic media: Czapek, SM-1, SM-III starch-ammonium agar, also potato agar, wort agar, etc. Of the deficient media we used tap water + 1.5% agar-agar, semideficient media of Ashby, SM-1 and others.

We studied 24 strains. One strain, No. 24, ceased to grow in subculture and perished. Strain No. 22 represents a proactinomycete, and was not correlated with the other strains in the general analysis.

Each strain was subjected to 2-3 parallel tests, while doubtful strains underwent 4-5 parallel tests. All cultures were analyzed three times according to their growth stages, i.e. on the 10th, 15th, and 20th days. Hence, each strain was examined microscopically 10-15 times, some even more. The results are given in Table 3.

It can be seen that 12 strains, Nos. 4, 5, 8, 9, 11, 12, 13, 19, 20, 21, 23 and 25 have spiral sporophores in almost all the media tested, the spirals being well pronounced, numerous

in each culture, with 2 to 5 or more coils. They may be extended or tightly compressed. These data agree with those of most other foreign laboratories. All these strains clearly belong to spiral forms.

Strains No. 14, 17 and 18 cause no difficulties. They do not form spiral sporophores in any of the test media. Their sporogenous branches are straight or flexuous, short or long, without any spiral coils. Many foreign investigators also have noted the absence of spirals.

The remaining 8 strains are less well defined, and the data on them most contradictory. Therefore we centered our attention on these strains and studied them most thoroughly. Each strain was subjected to special analysis and detailed microscopic examination. Finally we established that all these cultures belonged to the class of actinomycetes with scant fructification. They very rarely form spiral sporophores, and only in individual cultures single spirals can be detected after a long examination of the Petri-dish with the culture.

Strain No. 1 showed individual spiral with 1-3 coils only on two media: in the Czapek medium with maltose and in a starch-ammonium agar. In strain No. 7 such a spiral formation was observed only in one medium, starch-ammonium agar. In strain No. 10 spirals with complete coils of 1-3 turns were noted only on SM-1 medium with sucrose. The spirals were very rare and could not always be detected. Most frequently the sporophores terminated in incomplete spiral coils of 0.5-1 turn. Such sporophores often form in other tested media.

In strain No. 15 individual spiral sporophores are found on a CPI medium with sucrose. On the other media the sporophores are nonspiral, long curved or flexuous, but not coiled into spirals.

In strains No. 2, 6 and 16 the sporophores terminate in an incomplete spiral or a coil of 0.5-1 turn. Such formation of sporophores is revealed in many media and is rather abundant. Our attempts to obtain real spiral sporophores in these strains failed.

Strain No. 3 does not form spiral sporophores on any of the media tested. It has long and drastically curved sporogenous branches which often resemble spiral coiling; we did not, however, observe real spirals. It is probable that some investigators took such curved branches for spirals.

Table 3. Formation of spiral sporophores in actinomycetes on various media (International Collection). Author's observations.

Strain No.	Water-agar	Czapek medium with sucrose	Czapek medium with glucose	Starch-ammonium	SM-1 with sucrose	Potato agar	Meat peptone	Synthetic (Subcommittee)
1	-	-	+	+	-	-	-	-
2	-	-	-	+	-	-	-	-
3	-	-	-	-	-	-	-	-
4	+++++	++++	+++++	+++++	+++++	++++	+	+++
5	+++++	+++++	+++++	+++++	+++++	+++	++	+++++
6	+	-	+	+	-	-	-	-
7	-	+	-	++	+	-	-	-
8	+++++	+++++	+++++	++++	+++++	++	+	++
9	+++++	+++++	++++	+++++	++++	++++	++++	+++++
10	-	++	+	+	++	+	-	+
11	+++++	+++++	++++	+++++	++++	++++	-	+++
12	++++	+++++	+++++	++++	+++++	++++	+	++
13	++++	++++	++++	+++++	-	+++	-	++++
14	-	-	-	-	-	-	-	-
15	-	-	-	-	+	-	-	-
16	+	+	+	-	+	-	-	-
17	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-
19	++++	++++	++++	+++++	++++	++++	++	++++
20	+++++	+++++	+++++	+++++	++++	+++++	++	++++
21	++++	+++++	++++	+++++	+	++++	+	++
22	-	-	-	-	-	-	-	-
23	++++	+++++	++++	++++	++++	+++++	+++	+++++
25	++++	++++	+++++	+++++	++++	++++	+	+++

Thus, the study of the international collection of actinomycetes can be summed up as follows: of the 24 strains investigated by us 19 represent organisms with spiral sporophores, 4 constitute the group with nonspiral sporophores and 1 is a proactinomycete.

Among the first 19 strains 12 have abundant complete spiral sporophores produced in all or most media, 4 strains (1, 7, 10 and 15) very rarely form spiral sporophores with 1-3 coils and even then not on all media; 3 strains 2, 4, 16) have incomplete spirals with 0.5-1 coil on the sporophore end.

Among the 4 strains of the second group (with straight sporophores), strain No. 3 has curved but nonspiral sporophores. In cursory examination they may be taken for spiral forms.

Spore Structure in Actinomycetes

The structure and form of spores are an essential character in actinomycetes. We have reported earlier that spores in actinomycetes may be of dual structure. In some cases they are spherical and oval in shape; in others rod-like and cylindrical.

Further investigations have shown that actinomycetes with spherical and oval spores are found more often. Not infrequently strains with spherical spores simultaneously have elongated or even rod-like spores. At times the same sporophore branch bears both spherical and elongated spores. Such spores are observed in many representatives of the globisporine group and even in one and the same species. For instance, A. streptomycini may exhibit spherical, oval and elongated spores at the same time. There are cultures, however, whose spores have only a rodlike or cylindrical shape. In such cultures this character is rather constant and may serve in the differentiation and identification of the species.

The mode of spore formation is an essential character. Previously we showed that cultures of some actinomycetes form spores by fragmentation, in others by segmentation. In the first case the protoplast of the sporogenous branch breaks up into separate sections, exhibiting a chromatinous substance and protoplasm strongly stained by basic dyes. These sections become compacted, rounded up and develop

into spores. The hyphal wall does not disappear. The spores are inside the hypha. Later on the hyphal wall dissolves or bursts and the spores are released (Krasil'nikov, 1938).

In segmentation, spores are formed by simple division of the sporogenous branch with the aid of cross partitions. The latter are formed in a usual way simultaneously along the whole sporophore. Then such a hypha breaks up into separate cell-spores. In such cases the newly formed spores have a cylindrical shape with sharply cut ends. Later they may round off. If the spores are short, they become oval or spherical; if the spores are long, they become elongated with rounded ends. Consequently, when determining the shape of the spore, one should know their original shape immediately after the breakup of the sporophore.

Cultures forming spores by segmentation are found rarely and it is only natural that they are not so well investigated as cultures with fragmentation spores. Our data indicate that such organisms should represent special species or groups of actinomycetes. It is worth mentioning that segmentation spores are typical of many proactinomycetes. In this respect, the actinomycetes show great similarity to proactinomycetes. As shown by our investigations, this similarity is not only external, but also phologenetic.

The relationship between the actinomycetes and the proactinomycetes was established by experimental variability (Krasil'nikov, 1939) as well as by finding intermediate forms in nature. We described strains which could be equally classed as actinomycetes or proactinomycetes on the basis of their structure and development.

In recent years much attention has been given to the structure of the spore wall. In electron microscope investigations, Kriss et al. (1945), Carvajal (1946), and Bringman (1951) found the surface of actinomycete's spores to be smooth and not exhibiting any outgrowths.

More recently Flaig (1952), then Küster (1953), Nikitina (1957), Ettlinger et al. (1958), Baldacci (1953-60), and others established that actinomycetes may have spores with a smooth membrane and spores with outgrowths. These outgrowths may be of different types: warty, prickly, needle-like, filamentous; they may also be long or short.

Some investigators are convinced that these outgrowths are sufficiently characteristic to be used for classifying actinomycetes. They established special sections on the

basis of the shapes of these outgrowths (Ettlinger et al., 1958; Flaig and Kutzner, 1958; Preobrazhenskaya et al., 1959). The sections established by them included variegated species, often differing greatly. For instance, in the same section Ettlinger includes the producers of streptomycin, A. streptomycini, A. aureus, A. candidus, A. coelicolor, A. ruber, A. olivaceus. According to Preobrazhenskaya (1959) this procedure is unsuitable for grouping actinomycetes. The material presented by them shows that the same series includes cultures with smooth, prickly, hairlike and needlelike outgrowths. The resumé, however, presents an opposite conclusion.

Analyzing the published data and our own observations, we may consider two types of spores: smooth and prickly, the same strain may have different outgrowths: prickly, needlelike, hairlike and tuberclelike, depending on the conditions of culture growth.

In studying our collection of actinomycetes Leshevalier and Tihonienko (1960) showed that in some cases individual smooth spores are found along with prickly spores in the same preparation and on the same sporophore. However, these individual spores are an exception.

The cultures studied show a definite relationship between the shape of the spores and their species group. Nikitina (1957) established that actinomycetes of the globisporus group have smooth spores. In the violet group we have studied 45 strains belonging to several different species. In one species (3 strains) the spores had a smooth surface, in all others (39 strains) they proved to have outgrowths.

We noted prickly spores in some species of blue actinomycetes, one species of the green group. A. viridis, also in A. viridichromogenus; in actinomycetes of the orange group (35 strains, 5 species) we observed spores with a smooth membrane.

At this juncture we cannot tell whether such a relationship between the spore shape and the species group is inherent in all actinomycetes. The data available, however, point to the fact that this character may serve the purposes of differentiating the organisms under study. Yet, it is suitable only for subdividing the species within definite, outwardly uniform groups, rather than accidentally selected sections or series.

Leshevalier and Tihonienko have shown that this character

is stable, it is manifested under various growth conditions, in various media. They tested 29 media in which they cultivated several strains of various actinomycete species. The spores were investigated at different stages of growth and development of the cultures after 3, 10, 16 and 20 days. Prickly spores were found in A. coelicolor (strains 1047, 2078), A. violaceus (strains 829, 1144, 2525, 2522, 2069, 2587, 117, 719) and A. viridichromogenus (strains 93 and 94).

Their data show that the spore surface does not change with the composition of the medium: in strains with smooth spores the latter remain unchanged, and prickly spores retain the prickles under different conditions of nitrogen and carbon nutrition. Only in individual cases have they observed spores with a smooth membrane along with prickly spores.

The authors managed to trace the sequence of the formation of prickles on the membranes. According to their data, the prickles form after the spores have acquired a sufficiently definite shape and have begun to ripen. Our observations show that they form at the expense of the external layer of the wall.

The material presented here gives us grounds to believe that the presence or absence of outgrowth on the spore surface may be used as a differentiating character when grouping actinomycetes. Two types of spores may be regarded as established: smooth spores and spores with prickly and other outgrowth.

Assuming the external structure of the spores to be a taxonomic character, we can use it only in combination with other characters when identifying and establishing actinomycete species. This character can be used successfully when distinguishing species within outwardly uniform groups.

On Verticils in Actinomycetes

The tables recommended by the Subcommittee include characters relating to the types of arrangement of sporogenous branches. They single out monopodial sporophores, i.e. those alternating on the mycelium filaments; followed by the verticillate arrangement, the verticils being subdivided into primary and secondary. The sporophores in these verticils may be straight or spiral. Thus, 5 types of formation arrangements of sporophores are differentiated. Data on the arrangement of the sporophores are presented

in Table 2. They are less contradictory than the data on the structure of sporophores and spores.

It should be noted that this character cannot as yet be used to differentiate species or groups of actinomycetes. It is still far from clearly understood and we cannot state the degree to which it is stable and hereditarily fixed. Our observations of many years standing show that almost all (or even all) cultures can form verticils under certain conditions of growth and in certain nutrient media. In some groups, however, verticils are found more often and more consistently than in other representatives of the actinocetes. For instance, verticils are usual in orange actinomycetes, they are formed on many or all media where air mycelium is present. Less frequently, they are observed in actinomycetes of the red and yellow-brown groups. They are rare in the cultures of the grey, white, blue and other groups.

The nature of the branching and arrangement of the sporophores may be used only for additional characterization of the strains, rather than for the determination and identification of the species.

All these data are of a very relative nature. Orange actinomycetes can develop in media without the formation of verticils, or the latter may be detected rarely, as in many grey or white actinomycetes.

Culture Colouration

As is well known, cultures of many actinomycetes display different colours. Colouration is characteristic of aerial mycelium, colonies and nutrient media. Not infrequently colouration affects only aerial mycelium, or, more precisely, spores, whereas colonies and media remain colourless. Aerial mycelium in pigmented cultures often differs in colour from those of colonies or of media. For example, in blue actinomycetes aerial mycelium may have whitish-grey, light grey, bluish, greenish-blue, bluish-grey, bluish-brown or some other colour. The colonies and the medium are usually blue or dark blue in colour. An acidified medium turns red. Pigments in this actinomycetes group belong to the anthocyanin type; as litmus, they change their colour with change in the pH of the medium.

Culture pigmentation is an essential and highly expressive

taxonomic character, and all investigators attach much importance to it. Some authors, however, attach more importance to the colouration of aerial mycelium and often class actinomycetes into sections and series according to this characteristic (Baldacci et al., 1953; Hesseltine et al., 1954; Flaig and Kutzner, 1958; Yamaguchi and Saburi, 1955; Ettlinger et al. 1958; and others). In the USSR a group of workers of the Prof. Gauze Laboratory differentiates actinomycetes by the colouration of air spores (see Collection of articles on classification of actinomycetes, 1957).

The Subcommittee recommendations quote the definite shades of the colouration of aerial mycelium and the colony (substrate mycelium). There is a great divergency in the data on these characters presented by different laboratories; the discrepancies are particularly pronounced in the data on the colouration of aerial mycelium. There is not a single strain with identical colour characters; 5 or 6 colours are given for each strain, certain strains showing 8-9 colours of the aerial mycelium. For instance, data of 118 analyses performed by different laboratories are presented with respect to strain No. 12. Of these, 27 characterize aerial mycelium as white, 1 as yellow, 1 as orange, 9 as pink, 3 as red, 3 as green, 60 as grey, and 11 as brown; i.e. here we have almost all possible colours. Similar diversity is observed in the remaining strains. Table 4 indicates aerial mycelium colouration for 17 strains; the colourations of the other strains coincide with those of the neighboring cultures.

The Subcommittee gives quite vague indications regarding the colouration of substrate mycelium (colonies). The Subcommittee table (Table 5) quotes colours; white, grey, colourless (colonies), then yellow, orange, red, violet, blue, green, brown and black. Nearly all strains were assigned 5-6 colours of the colonies. For instance, 121 analyses of strain No.1 yielded in 18 cases colourless colonies, in 30 white, in 33 yellow, in 4 orange, in 7 grey, in 29 brown. For strain No. 11 the colour of the colonies is given: as colourless in 13 cases, as white in 18, as yellow in 24, as orange in 5, as red in 1, as violet in 4, as green in 1, as grey in 17, as brown in 34, and as black in 1. Similar diversity is observed with the other strains.

Having analyzed the tables presented by the Subcommittee and the results of our own investigations, we come to the conclusion that the diverging indications noted by different

Table 4. Colouration of aerial mycelium of cultures from the international collection based on data from various laboratories.

Strain No.	White	Yellow	Orange	Pink	Red	Blue	Green	Grey	Brown
1	78	4	-	3	-	-	-	-	3
2	64	22	-	-	-	-	3	24	6
4	32	4	-	12	6	-	-	54	11
6	17	19	-	2	-	-	2	66	11
7	31	9	1	2	-	-	-	54	5
10	35	2	6	36	7	-	-	4	31
12	27	1	1	9	3	3	3	60	11
13	10	-	-	-	-	27	37	51	2
14	51	30	-	25	4	-	4	1	-
15	36	7	1	-	1	6	1	40	2
16	26	45	-	-	-	-	4	15	1
17	62	21	1	-	-	-	8	10	11
19	40	-	-	48	17	5	-	3	-
20	15	2	-	-	-	35	37	42	-
21	13	1	-	-	-	27	32	54	2
23	34	3	-	6	-	1	1	45	13
25	40	1	2	24	4	-	-	32	8

authors' with regard to the pigmentation of the colonies are the result of a subjective evaluation and often give an erroneous characterization of the colour.

It serves no purpose to subdivide the actinomycete colonies into colourless, white and grey. There can be no such differentiation. A colony of an actinomycete (substrate mycelium) can never be white or grey. It can be colourless, i.e. having no special colour. Greyish, or more precisely, dingy colonies are typical of all colourless actinomycetes. they may be more or less bright, depending on the composition of the medium. Our observations show that colourless, white and grey colonies are of the same order of colouring.

Table 5. Colour of actinomycete colonies from international collection on the basis of data from various laboratories.

Strain No.	Number of analyses	Colourless	White	Yellow	Orange	Red	Violet	Blue	Green	Grey	Brown	Black
1	112	18	30	33	4	-	-	-	-	7	29	-
2	103	3	3	43	2	-	-	-	1	-	47	4
3	156	-	7	44	-	1	-	-	31	28	21	21
4	132	21	32	10	6	19	-	-	-	25	19	-
5	131	-	1	24	8	10	-	-	6	12	69	-
6	93	-	-	67	8	1	-	-	3	2	12	-
7	114	14	20	38	6	3	-	-	2	16	13	-
8	123	20	34	42	2	-	-	-	-	19	6	
9	132	11	25	52	14	-	-	-	-	3	27	-
10	117	28	21	14	9	8	1	-	-	8	28	-
11	123	23	18	24	5	1	4	-	1	17	34	1
12	119	8	15	8	4	5	4	-	1	19	55	-
13	154	-	-	-	-	-	-	3	43	32	31	45
14	123	-	2	42	49	27	3	-	-	-	-	-
15	92	29	10	3	1	-	-	3	2	28	11	5
16	111	34	5	26	-	-	-	-	6	10	14	16
17	109	13	12	57	1	-	-	-	2	3	21	-
18	116	19	6	10	-	3	23	4	-	21	30	-
19	97	-	2	1	-	51	44	-	-	3	-	-
20	112	6	7	56	9	1	-	-	2	4	27	-
21	127	3	1	-	-	-	2	3	27	29	39	23
23	91	-	1	24	2	-	-	-	-	-	60	4
25	103	3	14	3	24	41	-	-	-	4	14	-

Quite often colourless colonies have a yellow or brownish appearance on the lower side (yellowish-brownish lining). Such cultures have no real pigmentation, being, in fact, colourless forms.

It is entirely improbable that colourless cultures should have such diverse pigmentation as that shown in the table. When noting some culture colouration or other, investigators do not attempt to establish whether it is due to a real pigment or to secondary substances of nonpigmentary nature. Only such colony colouration is suitable for the purposes of taxonomy and differentiation of species which is due to a real pigment, i.e. a metabolite with definite staining and physico-chemical properties.

The colouring of culture-colonies and aerial mycelium varies markedly depending on the composition of the medium and the conditions of growth (Krasil'nikov, 1938, 1949-60). It is most characteristic and pronounced in synthetic media. In these media pigments have a colouring without impurities (brown, chestnut, yellowish, etc.). In complex and especially in albuminous media pigments are less pronounced; often they do not manifest themselves at all or mix with various supplementary substances which alter the basic colour of the pigment.

In certain species pigmentation is particularly pronounced when individual elements are added to the medium. For instance, in actinomycetes of the green group the pigment is very prominent in the presence of iron citrate. In these cultures iron strongly stimulates the synthesis of the green pigment.

In blue actinomycetes which have, for one reason or other, lost their ability for synthesizing pigment, biotin, if added to the medium, completely restores it, and in weakened strains pigmentation markedly increases. In violet strains with weakened pigmentation the synthesis of the pigment is enhanced under the influence of vitamin B_{12} (Krasil'nikov and Egorova, 1960).

Rather often actinomycete cultures exhibit pigmentation most vividly in synthetic media with an addition of extracts from yeast or corn, oatmeal and other vegetative residues.

We carried out a series of special experiments with a view to establishing the colouring of the actinomycetes studied. The latter were grown on different nutrient media; simple synthetic and complex organix, albuminous: the

Czapek medium with glucose and sucrose, starch-ammonium, glycerol-asparagine, etc., then media with an addition of oatmeal and corn flour, a medium with a yeast extract, with meat-infusion broth, etc.

Our investigations have shown that there are 6 colourless strains in this collection (Nos. 1, 8, 9, 10, 17, 20) and 3 doubtful strains (11 and 12). They are being subjected to further study. One strain (No. 2) belongs to the globisporus group, it is also colourless. Three strains (6, 20, 23) belong to the group of fluorescent actinomycetes, they form a lemon-yellow pigment diffusing into the medium. Two strains (13, 21) have a brown-green pigmentation of the colonies and the medium, and two (3, 5) have a doubtful similar colour. They stand closer to the group of brown-green actinomycetes. Two strains (7, 16) have a definitely yellow colouring of the colony, not of the medium; two have a red-orange or red-pink colour (4, 14); two have a blue colour (15, 18) and two have a violet pigmentation.

To sum up, we can say that among the external morphological and cultural characters, the former are most significant, namely the structure of sporophores and spores. Only such culture colouring can be used successfully which is due to real pigments not to secondary accidental products of metabolism. The most stable pigmentation is that of substrate mycelium (colony). Of essential significance for the differentiation of actinomycete cultures is the ability of pigments to diffuse into the medium. This gives some idea of certain physico-chemical properties of pigment substances.

Less reliable is the colouring of aerial mycelium. However, this too, may be of some significance in differentiating actinomycetes; in individual cases it is sufficiently characteristic of certain groups. For instance, the green colouring of aerial mycelium is inherent only in definite species.

No matter how essential morphological or cultural indications may be, they can not serve as the sole basis for building up the taxonomy of organisms. As we repeatedly emphasized (1939-60), the classification and taxonomy of actinomycetes should be based on a combination of characters: morphological, cultural and physiologic-biochemical. Of great importance in the differentiation of species and, consequently, in taxonomy, are antimicrobial properties and the specificities of their antagonisms (Krasil'nikov, 1950-60).

The structure of sporophores and spores, as well as culture pigmentation, each taken separately, cannot characterize species since these indications are too general. One and the same structure type of sporophores or spores is characteristic of many entirely different species.

Spiral sporophores are found in representatives of blue, violet, yellow, colourless and other groups of actinomycetes. They cannot be combined into a single species or into a single group by the type of sporogeny.

The same is true of pigmentation as a character. One and the same type of pigment, one and the same culture colouring may be encountered in different actinomycete species.

We agree with Baldacci that the pigmentation of cultures, notably that of vegetative mycelium (colonies) and the medium may be useful in the preliminary, very tentative grouping of actinomycetes, but not in establishing taxonomic units.

In our first manual of ray fungi (1941) the cultures are arranged in a definite order by groups in accordance with their pigmentation: starting with blue actinomycetes followed by violet, red, yellow, green, orange, etc. Having no intention to assign taxonomic significance to this group, we did not dare then to use the term "group" to denote the above-mentioned sections. We did not supply the corresponding headings.

At present considerations of purely practical convenience make us use the terms "group" and "subgroup" for certain cultures having identical pigmentation indices and the structure of sporophores and spores. We do not yet assign these groups the significance of a species, subspecies or superspecies, or larger taxonomic unit. A group of outwardly uniform strains may include a few species differing in biochemical, physiological and antimicrobial properties and the specificity of antagonism.

Groups are built, not according to a single character, but according to their combinations. These characters may be different from case to case. For instance, in colourless actinomycetes having white aerial mycelium we observe a group with straight sporophores and a group with spiral sporophores. Green, violet and blue groups of actinomycetes are formed by the solubility of the pigments and by the nature of fructification. It is quite possible that there

are also other external characters which might be used for the same purpose. Further investigations should be carried out with the aim of establishing new groups and their species. All strains should be thoroughly studied in each group.

After a careful study we classified the globisporus group into 8 species, in violet actinomycetes we detected 7 species and 2 subspecies, the white-blue group clearly exhibits 2 species, the brown-green group also 2 species, the fluorescent group 3 species, etc. (see collection of papers "Biology of Individual Groups of Antibiotic-Producing Actinomycetes 1960").

The actinomycete group enables one, using the uniformity of external indications as a guiding principal, to make a clearer distinction of the great diversity of cultures isolated from natural substrates.

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EDITORIAL NOTE:

The illustrations to accompany Dr. Krasil'nikov's article (this Bulletin pp. 133 to 159) have been delayed in the post. They will be published in a later issue.

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
Volume 11, No. 4 October 15, 1961 pp. 161-164

PROPOSAL FOR DESIGNATION OF NEOTYPE STRAINS OF LEPTOTRICHIA BUCCALIS AND BACTERIONEMA MATRUCHOTII

To: Judicial Commission
International Committee on Bacteriological Nomenclature of the
International Association of Microbiological Societies
Attention: Dr. R.E. Buchanan, Chairman

As noted in the accompanying reprint*, for many years there has been considerable confusion in the nomenclature of certain Gram-positive, rod-shaped or filamentous bacteria isolated from the oral cavity. As a result, two entirely different organisms have been included in the genera Leptothrix and Leptotrichia. To clarify this confusion the following proposal is offered.

1. Robin's name, Leptothrix buccalis Robin 1853, was invalid. (see Buchanan, Jour. Bacteriol. 3:301-306, 1918.)

2. Trevisan's proposal of the genus Leptotrichia (1879), with L. buccalis Trevisan as the type species, for certain of the filamentous forms of the oral cavity was both useful and valid. However, he failed adequately to characterize the genus, and gave no description of the type species.

   A. It must be assumed that his type species had characteristics which conformed to those given for the genus. (See 6A below.)

3. Trevisan's inclusion of Leptothrix buccalis Robin and Lebert as a synonym of his Leptotrichia buccalis was an error.

*Gilmour, Marion N., Arden Howell, Jr., and Basil G. Bibby. 1961. The classification of organisms termed Leptotrichia (Leptothrix) buccalis. I. Review of the literature and proposed separation into Leptotrichia buccalis Trevisan 1879 and Bacterionema gen. nov. (Mendel 1918) comb. nov. Bacteriol. Rev. 25:131-141.

A. The reference given was incorrect, since the name Leptothrix buccalis was first used by Robin alone in 1853.

B. Trevisan's definition of the genus Leptotrichia did not correlate with Robin's description of Leptothrix buccalis.

4. DeToni and Trevisan's later removal of Leptotrichia buccalis Trevisan to the new genus Rasmussenia as R. buccalis was illegitimate.

A. A new genus cannot contain the type species of a previously described genus which was legitimate.

5. DeToni and Trevisan were in error in using Leptothrix buccalis Robin and Lebert as a synonym of Rasmussenia buccalis.

A. The name Leptothrix buccalis was first used by Robin alone in 1853, not 1847.

B. The DeToni and Trevisan description of Rasmussenia buccalis does not correlate with that given by Robin for Leptothrix buccalis.

6. It must be assumed that DeToni and Trevisan's definition of Rasmussenia buccalis applied to Trevisan's earlier Leptotrichia buccalis for which no description was given.

A. This definition, though inadequate, correlates more closely with his earlier generic definition of Leptotrichia than does that of Robin for Leptothrix buccalis.

7. Opinion 13 of the Judicial Commission is hereby revised by the adoption of Leptotrichia buccalis Trevisan 1879 as the type species of the conserved genus Leptotrichia rather than Leptotrichia buccalis (Robin) Trevisan.

8. Culture No. 14201 of the American Type Culture Collection is recognized and approved as a neotype culture of Leptotrichia buccalis Trevisan.

A. To date no other culture has been designated as a type of neotype strain. This strain conforms to the description of the venus Leptotrichia Trevisan 1879 and to some extent to that of Rasmussenia buccalis DeToni and Trevisan 1889.

B. This culture also conforms to the description of the organisms studied adequately by Thjøtta et al. (1939) for which they proposed the name Leptotrichia buccalis Trevisan 1879.

9. Culture No. 14266 of the American Type Culture Collection is recognized and approved as a neotype culture of Bacterionema matruchotii (Mendel, 1919), comb. nov., Gilmour, Howell, and Bibby, 1961, the type species of the new genus Bacterionema.

A. This culture correlates with the description of the organism studied and described by Kligler, 1915, as Leptothrix buccalis.

Respectfully submitted,

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INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
Volume 11, No. 4 October 15, 1961 Page 165

NOTICE

To Members of the International Committee on Bacteriological Nomenclature of the International Association of Microbiological Societies

Meetings of the International Committee on Bacteriological Nomenclature will be held on days immediately preceding and during the VIII International Congress for Microbiology 19-25 August 1962 to be held under the auspices of the I.A.M.S. and of the Canadian Society of Microbiologists at Montreal, Canada. Headquarters will be the Queen Elizabeth Hotel.

The members of the International Committee are urged to make necessary reservations and secure details of registration through Secretary General N. E. Gibbons, National Research Council, Sussex Drive, Ottawa 2, Canada.

The Committee has a membership of more than ninety individuals representing Microbiological Societies in more than thirty nations. Each nation should be represented. If it is not possible for any member to attend, it is urged that a satisfactory alternate be nominated to one of the Joint Permanent Secretaries.

The Committee will receive a long list of agenda to be considered, including many recommendations from the Judicial Commission. It is hoped that most of the work of the Committee may be completed on Friday and Saturday 17-18 August. Objections to Opinions issued by the Judicial Commission and numerous amendments to the International Code of Nomenclature of the Bacteria and Viruses will be considered.

E.G.D. Murray,
Chairman
S.T. Cowan,
Joint Permanent Secretary
W.A. Clark,
Joint Permanent Secretary

NOTICE

To Members of the Judicial Commission
of the
International Committee on Bacteriological Nomenclature

Meetings of the Judicial Commission of the International Committee on Bacteriological Nomenclature will be held on days immediately preceding and during the VIII International Congress for Microbiology 19-25 August 1962 to be held under the auspices of the Canadian Society of Microbiologists at Montreal, Canada. Headquarters will be the Queen Elizabeth Hotel.

The members of the Judicial Commission are urged to make necessary reservations and secure details of registration through Secretary General of the Congress, N.E. Gibbons, National Research Council, Sussex Drive, Ottawa 2, Canada.

The Commission will have a long list of agenda to be considered. It is hoped that most of the work of the Commission can be completed on Thursday, Friday and Saturday 17-18 August. The Congress opens Sunday 19 August. A list of the agenda and times and places of the meetings of the Commission will be published in later issues of the Bulletin.

Will members of the Commission who find it impossible to attend the meetings notify the Chairman and either designate an alternate or authorize the joint secretaries to appoint an alternate?

Many Opinions and amendments to the Code of Nomenclature will be considered. A full attendance is desired.

R.E. Buchanan,
Chairman
S.T. Cowan,
Joint Permanent Secretary
W.A. Clark,
Joint Permanent Secretary

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
Volume 11, No. 4 October 15, 1961 pp. 167-168

ORGANIZATION OF A COMMITTEE ON THE NOMENCLATURE OF PATHOGENIC BACTERIA IN THE MINISTRY OF PUBLIC HEALTH OF THE U.S.S.R.

NOTE: The following statement is taken from the English translation (Jour. Microbiol., Epidemiol., Immunobiol. 31(8):1573. 1960)* by Dr. D.B. Rolph, and is here reproduced because of its interest in systematic bacteriology.

- - - - - - - -

Investigations into the question of classification of microorganisms are conducted over the years in live culture collections, in scientific and practical scientific institutions working in the field of microbiology, virology and parasitology. Classifications put forward by institutions or by individual scientists require detailed investigation and approbation. Preliminary confirmation by an official Soviet body is also needed for introduction to discussion in the International Nomenclature Committee. However, so far such a body does not exist. By an order of the Ministry of Public Health, U.S.S.R. No. 378 of 14 August 1959, a Committee was set up for the Nomenclature of Pathogenic Bacteria in the State-Controlled Tarassevich Institute of Medical Biological Preparations for the approbation of the classification and nomenclature of bacteria, viruses and bacterial preparations under the chairmanship of Active Member of the Academy of Medical Sciences U.S.S.R. Professor V.M. Zhdanov. The Committee was charged with the examination and confirmation of the systems of classification of pathogenic bacteria, viruses and protozoa, and their agreement with the International Code, the conferring again of names upon newly discovered bacteria and viruses, and also newly proposed biological preparations and a series of other questions connected with the classification and nomenclature of bacteria, viruses and the protozoa.

The body of the Committee consists of 17 specialists in different fields of microbiology, virology and parasitology; on 30 November 1959 the constitution of the Committee for

* Russian: Zh. mikrobiol. epidemiol. immunobiol. 31(8): 143, 35. 1960.

the nomenclature of pathogenic bacteria of the Ministry of Public Health, U.S.S.R. was confirmed. According to this constitution the plenary sessions meet 4 times a year; the permanent leader of the work of the Committee takes office.

The first plenary session took place on 25 February 1960. Besides general questions on organization a lecture was heard at the first meeting by Professor E. M. Novgorodska "On the question of classification of the dysentery bacilli." The speaker presented summarized data on the work over several years of the Department of Intestinal Infections of the Leningrad Pasteur Institute of Epidemiology, Microbiology and Hygiene on the question of the classification of the dysentery bacilli and also the results of a comparative investigation of the morphological and biochemical properties and of the antigenic structure of collections of type cultures of dysentery bacilli according to the provisional Soviet classification and of reference strains of the genus Shigella received from the British collection. After an animated exchange of opinions the following resolutions were adopted:

1) to change to the international classification in the designation of the types of dysentery bacilli;

2) to carry out preliminary work on the question of the division of organisms of the genus Dysenteriae [sic] into species, subspecies and subtypes;

3) to entrust the carrying out of this work to a Commission of specialists;

4) on the basis of the material worked out by the Commission, to send a series of articles on these questions to the International Bulletin of Taxonomy, and to submit comparative proposals to the International Committee on Nomenclature.

Z. M. Andreeva

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